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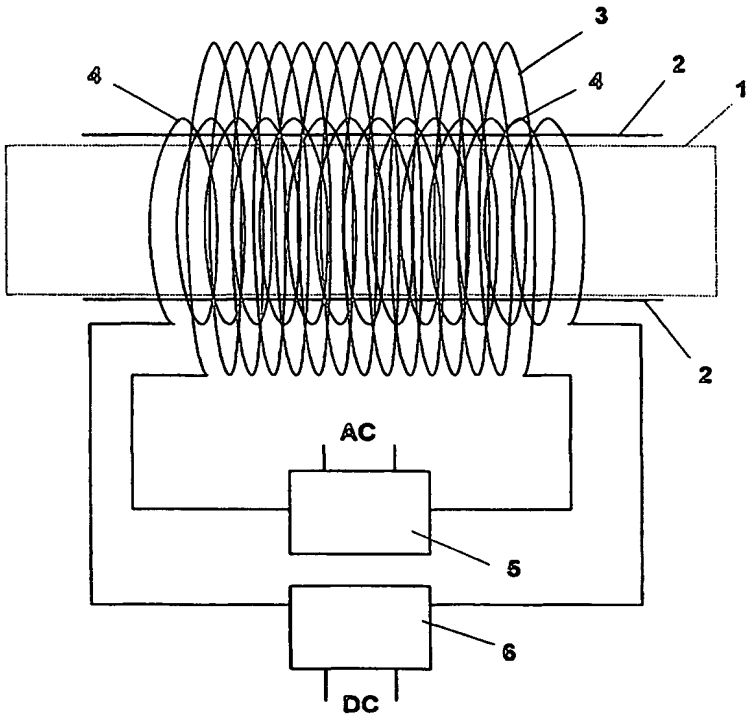
(57) Abstract

A method and an apparatus for interfering with pathological cells survival processes, i.e. inducing directly or indirectly apoptosis, on living pathological cells, by using magnetic fields without adversely affecting normal cells. Static (S) and extremely low frequency (ELF) magnetic fields are used having low intensity comprised between 1 and 100 mT, preferably comprised between 1 and 30 mT. In particular SELF fields are used which are different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF fields together, as well as the presence of S or ELF fields alone, said ELF fields having a field frequency comprised between 1 and 1000 Hz. An apparatus for carrying out the method comprises means for generating static magnetic (S) fields crossing a working environment and/or means for generating electromagnetic extremely low frequency (ELF) fields over the working environment in addition to the S fields. Means are provided for mudulating the S fields associated to the S fields generating means and varying the intensity of the S fields from 1 to 100 mT, preferably between 1 to 30 mT according to a predetermined function. Means may also be provided for modulating the ELF fields associated to the ELF fields generating means and imposing to the ELF fields a frequency between 1 and 1000 Hz with intensity comprised between 1 to 100 mT, preferably between 1 and 30 mT according to a predetermined function.



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TITLE

APPARATUS AND METHOD FOR INTERFERING WITH PATHOLOGICAL CELLS SURVIVAL PROCESSES

DESCRIPTION

Field of the invention

The present invention generally relates to an apparatus for interfering with pathological cells survival processes.

In addition, the invention relates to a microbiological method carried out by such apparatus for interfering with pathological cells survival, in particular cells affected by cancer and other diseases caused by alterations in the mechanism of cell survival.

In particular, the interference is induced by means of static (S) and extremely low frequency electromagnetic (ELF) fields produced by the apparatus.

Magnetic Static fields and Extremely Low Frequency electromagnetic fields are hereinafter referred to also as S and ELF, respectively. Moreover, any possible combination of different sequences of S and/or ELF fields, such as S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, will hereinafter be referred to also as SELF fields.

Background of the invention

It is known that pericellular fields and currents induced by an Extremely Low Frequency (ELF) electromagnetic field, whose frequency range is from 1 Hz to 300 Hz and perhaps up to 1000 Hz, induce within the cell certain membrane electrochemical events which are important for primary biologic signal transduction and amplification processes.

These biochemically mediated events then produce cytoplasmic second messengers and internal effectors such

as free Ca^{++} and protein phosphorylases (kinases) which in turn trigger certain changes in the biosynthesis of macromolecules as well as bring about alterations in cellular growth differentiation and functional properties [1M. Blank, 1993].

Further, the possibility that S and ELF fields affect the DNA synthesis, DNA integrity, transcription and translation has been documented [2Liboff 1984, 3Tofani 1995, 4Goodman 1991, 5Phillips 1992].

A possible physical mechanism to account for some of the experimental findings is the direct effect on ions (i.e. Ca^{++}) or on ligand binding at the cell membrane [6Liboff 1985, 7Chiabrera 1985, 8Lednev 1991, 9Blanchard 1994].

The possibility of influencing variations of Ca^{++} metabolism may lead to cell apoptosis (programmed cell death) [10Preston, 11Trump 1997].

Another physical interaction mechanism is related to the possibility of influencing the kinetics of appropriate cell signalling pathways of the cell (including calcium metabolism) through a field direct effect on electron-spin motion of atoms and molecules with unpaired electrons. This influencing may affect the recombination ratio of a spin correlated free radical pair and consequently on redox signalling [12Grundler 1992; 13Polk 1992; 14Walleczek and Budingher 1992; 15Adey 1993].

In particular, the spin singlet-triplet energetic level transition in a free radical is critical for increasing the recombination ratio of spin correlated free radical pairs.

The possibility for low level, non thermal (with intensity up to 30 mT) S and ELF magnetic fields to influence in vitro the kinetics and efficacy of radical

pair reactions is known from magnetochemistry [[16]Steiner 1989].

Naturally occurring free radicals have an oxygen- or nitrogen-based unpaired electron such as superoxide anion, hydroxyl radical and nitric oxide. These Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) can target proteins providing an obvious mechanistic explanation for free radicals-mediated signalling events. These events may influence growth factors, ion transport (i.e. Ca^{++} channels), transcription, apoptosis [[17]Lander 1997].

Apoptosis is a morphologically distinct form of programmed cell death that is connected in cell survival processes playing an important role during development, homeostasis, and in many diseases including cancer, acquired immunodeficiency syndrome, and neurodegenerative disorders, as well as in other diseases that similarly to those are characterised by altered cell survival processes. Apoptosis occurs through the activation of a cell-intrinsic suicide program. The basic genetic mechanism of apoptosis appears to be present in essentially all mammalian cells at all times, but the activation of this suicide program is regulated by many different signals that originate from both the intracellular and the extracellular environment.

Among all the genes involved in apoptosis regulation, the p53 gene is receiving much attention. This gene, which encodes a transcription factor and is common in many human cancers, mediates the cellular responses to some environmental damage. The p53 protein either can temporarily stop cell division, so that the cell can repair altered DNA, or can pilot the cell to an apoptotic death.

Published data support that p53 appears in

apoptosis through a three step process: 1) transcriptional induction of redox-related genes: 2) the formation of reactive oxygen species and 3) the oxidative degradation of mitochondria components, culminating in cell death [[18]Polyak 1997] .

In addition anti-oxidative agents are combined with drugs in the treatment of hypoxia tumour cells [19] [Walch, 1988] and in the influence of vascular growth factor [20] [Amirkhosravi, 1998].

Moreover, published data are supporting the idea that pathological cells answer differently than normal cells to ELF fields stimuli. According to [21]Cadossi [1992], lymphocytes from normal patients respond differently than lymphocytes from Down's syndrome, AIDS and chronic lymphocytic leukaemia patients when exposed to ELF fields (previously with mitogen).

It is also recognised that Ca^{++} influx across the membrane is influenced by ELF fields in leukaemic lymphocytes but not in normal lymphocytes [[22]Walleczek, 1996].

Altered cell survival processes come with electric disorders and different electrical behavior. In fact, rapidly proliferating and transformed cells have electrically depolarized cell membranes if compared with normal cells [[23]Binggeli, 1986;[24] Marino 1994]. It has also been shown that epithelial cells lose their transepithelial potential during carcinogenesis [[25]Davies 1987; [26] Goller 1986; [27] Capko, 1996]. This different electrical behavior of tumor cells compared with normal cells is the basis for a newly proposed cancer diagnostic modality [[28]Cuzick 1998]. In addition, the concentration of free radicals in transformed cells and tissues is higher than in non-transformed ones [[29]Szatrowski 1991; [30] Shulyakovskaya 1993; [31] Iwagaki 1995].

With reference to chemotherapy all efforts are devoted to the target of inducing cell apoptosis in vivo instead of killing them, through Signal Transduction Directed Therapy (STDT) of cancer [[32]Levin, 1998].

Signal Transduction is a functional term that connotes the translation of genetic information into signalling cascades that allow the cell to for example interpret and respond to external stimuli and/or duplicate itself. Recent evidence suggests that alterations in the cell survival processes contribute to the pathogenesis of a number of human diseases, including cancer, viral infections, autoimmune diseases, neurodegenerative disorders, and AIDS. Treatments designed to specifically alter the apoptotic threshold connected with the survival processes mechanisms may have the potentiality to change the natural progression of some of these diseases [[33]Thompson, 1995].

High intensity electrical, electromagnetic and magnetic fields have been used to destroy pathological cells.

In [34]US4665898 an apparatus is described in which animals having malignant cells are treated by means of a high intensity pulsed magnetic field, in order to neutralise/destroy malignant cells in a selective way. This apparatus produces magnetic thermal fields having intensity comprised between 1 Tesla up to 10 Tesla and reversing polarity in the range 5÷1000 Kilohertz. In the preferred embodiment the magnetic field intensity is set between 1 and 50 Tesla and in particular, in the examples, it is set at 5 Tesla and 8 Kilohertz up to 18 Tesla and 250 Kilohertz.

Different ELF, thermal, continuous or pulsed fields, have been used for anti-cancer therapy in vitro [[35]Narita, 1997; [36]Raylman, 1996].

In these cases the fields are of very high intensity,

much higher than what people are allowed to be exposed by the safety standards, and may produce heating thus damaging normal tissues and cells.

ELF low intensity electromagnetic fields have been used as well to inhibit mitosis of malignant cells, such as in DE 4122380A1 and US 5156587. However, these documents describe the use of sinusoidal fields only at a fixed net frequency and at a fixed intensity, with the possibility to sweep only a limited range of energy levels inside the cellular tissue.

Summary of the invention

It is an object of the present invention to provide a method for interfering with cell survival processes (i.e. inducing apoptosis) of living pathological cells (i.e. cancer cells) by using magnetic fields without adversely affecting normal cells.

It is another object of the invention to provide an apparatus for interfering with pathological cells survival processes.

The former and other objects are reached by the method for interfering with pathological cells survival according to the invention whose characteristic is to apply to living pathological cells (i.e. cancer cells and cells affected by other diseases caused by alterations in the mechanism of cell survival) non thermal SELF magnetic fields to induce apoptosis in a selective way.

For the purposes of the invention SELF fields are to be considered as different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone.

The concept underlying the method according to the invention is that SELF fields interfere with cell signalling sustaining cell pathological behaviour inside

pathological cells, i.e. on redox signalling through free radicals, thus restoring the cell survival processes, i.e. inducing directly or indirectly apoptosis through a modification of p53 gene expression.

This method is supposed to recombine oxygen-based free radicals and may also be used as an anti-oxidative agent. It's combination with drugs in the treatment of hypoxia tumour cells and in the influence of vascular growth factor may also be considered.

The reason why SELF fields selectively induce apoptosis in pathological cells (i.e. cancer cells) may be related to the altered electrical behaviour of pathological cells compared with that of normal cells.

For these reasons SELF fields can induce directly or indirectly a signal programmed cell death (apoptosis), in vitro and in vivo, without causing any adverse effect.

In the hypothesis that free radicals recombination is at the basis of the expected biological effects on pathological cells (i.e., anti-tumour activity) the transition between singlet-triplet of unpaired electron in oxygen based free radicals has to be considered. In fact this transition, which depends on the applied magnetic field, is critical for increasing the recombination ratio of a spin correlated free radical pair. However, the reaction centres related to the expected anti tumor effect are unknown and therefore the lifetime of the spin states and the energy splitting between singlet and triplet states cannot be precisely determined from the spin hamiltonian [[37]Haberkorn 1979, [38] Lersch 1983].

To encompass this problem, according to the invention, sequences of S magnetic fields with different intensity modulated in amplitude can be used, with the superimposition of ELF magnetic fields. The use of modulated fields is in agreement with the need for

reaching optimal condition(s) for the singlet-triplet spin state conversion required for the free radical recombination processes [[13]Polk 1992].

For these reasons, S, ELF or SELF fields have higher probability to induce the expected biological effects if they are modulated following a predetermined function of intensity and or frequency versus time, since this way the probability to induce the above transition is higher.

The different sequences of S and/or ELF fields sequences are advantageously set for time intervals T_1, T_2, ..., T_n, wherein the intensity I_S, I_{ELF} and their ratio I_S/I_{ELF} are set at steady values I_{S1}, I_{S2}, ..., I_{Sn}; I_{ELF1}, I_{ELF2}, ..., I_{ELFn}, I_{S1}/I_{ELF1}, I_{S2}/I_{ELF2}, ..., I_{Sn}/I_{ELFn}, respectively.

For the same reasons modulated SELF non thermal fields can be potentially used for treatment of cells affected by many diseases like viral infections, AIDS, autoimmune diseases, etc., where the alteration of cell survival contributes to their pathogenesis.

According to another aspect of the invention, an apparatus for selectively interfering with pathological cells survival processes in vitro and in vivo has the characteristic of comprising means for generating static magnetic (S) fields crossing a working environment and means for generating electromagnetic extremely low frequency (ELF) fields in the working environment alone or in addition to the S fields.

Means are provided for modulating the S fields associated to the means for generating S fields and varying the intensity of the S fields between 1 and 100 mT and preferably from 1 to 30 mT.

Means are also provided for modulating the ELF fields alone or associated to the S fields at a frequency between 1 and 1000 Hz with intensity comprised between 1 and 30 mT. Preferably the ELF fields have a frequency

between 10 and 100 Hz.

In a particular embodiment of the invention the means for modulating the S fields comprises program means that alternatively or in combination:

- set the intensity following a plurality of predetermined step values I_{S1}, I_{S2},..., I_{Sn} for corresponding time intervals T_1, T_2, ..., T_n;
- set the intensity amplitude following a plurality of predetermined step values I_{ELF1}, I_{ELF2},..., I_{ELFn} for corresponding time intervals T_1, T_2, ..., T_n;
- set the frequency following a plurality of predetermined step values f_1, f_2,..., f_n, for corresponding time intervals T_1, T_2, ..., T_n;
- set an S/ELF ratio according to a plurality of predetermined step values I_{S1}/I_{ELF1}, I_{S2}/I_{ELF2},..., I_{Sn}/I_{ELFn}, for corresponding time intervals T_1, T_2, ..., T_n,.

Preferably, the program means set the S and ELF fields according to an overall intensity between 1 and 30 mT and respectively a ratio S/ELF comprised between 0,1 and 10 and, in a particularly preferred embodiment, according to an overall intensity between 1 and 10 mT and respectively a ratio S/ELF comprised between 0,5 and 5.

The time intervals are preferably set between 1 and 40 minutes.

At least a portion of the working environment is defined by walls permeable to the S and ELF fields. At least a portion of the working environment is also advantageously adjacent to a first and a second coil respectively and the means for modulating supplying to the coils DC and AC current respectively.

Brief description of the drawings

Several embodiments of the apparatus are shown in the attached drawings, given as an example and not limitative, wherein:

- Figure 1 shows a diagrammatical view of a first embodiment of an apparatus according to the invention;
- Figures 2 to 4 show block diagrams of a second third and fourth embodiment of an apparatus according to the invention, respectively;
- Figure 5A shows a diagrammatic function of field intensity versus time, as programmable in the apparatus according to the invention;
- Figure 5B shows a diagrammatic function of field intensity of S and ELF fields versus time varying also the ratio with respect to each other field;
- Figure 5C shows a diagrammatic function of field intensity and frequency versus time.

Description of the preferred apparatus

In figure 1 the working environment is indicated as 1 and the wall as 2. The first and second coils are given the reference numbers 3 and 4 respectively. The modulating means are diagrammatically indicated by boxes 5 and 6 respectively, and are connected to AC and DC sources.

In figure 2 a different embodiment of the apparatus, used for interfering with pathological cells survival both in vitro and in vivo has two coils 23 and 24 located coaxial to each other at the opposite sides of the working environment 21. Variable transformers 25 and 26 are provided connected to a 50 Hz AC electric network 27. Switchable diode bridges 28 are provided to change the AC supply to the coils. A DC transformer 29a, a rectifier 29b as well as a timer 29c are provided supplying two plates 29 so that an up to 20kV/m static (or low frequency variable up to 1000 Hz) electric field, and preferably about 6 kV/m, may be created in the working environment 21 within preferred intervals, according to the experimental conditions.

In figure 3 a further embodiment is shown of the

apparatus used for interfering with pathological cells survival in vitro having a SELF modulator 35 (1-100 Hz) and two coils 33 and 34 located coaxial to each other at the opposite sides of the working environment 31. An amplifier 36 is used between the modulator 35 and the coils 33 and 34, which are supplied with the same current creating in the environment 31 either an S or an ELF magnetic field.

Another embodiment of the apparatus according to the invention (fig. 4) used for interfering with pathological cells survival both in vitro and in vivo has two Helmoltz coils 43 and 44 located coaxial to each other at the opposite sides of the working environment 41. An amplifier 46 is used between the modulator 45 and the coils 43 and 44, through a shunt element 47, which is also connected to a personal computer 49.

Each apparatus can be used for producing SELF modulated non thermal fields for interfering with pathological cells survival.

With reference to figures 5A to 5C, an example of the programming of the apparatus is given wherein the modulation of intensity, frequency and intensity ratio between S and ELF fields is carried out.

In figure 5A the way in which the intensity I may vary versus time. I_1, I_2, I_3, I_n are the intensity or field strength (mT) of either the S field, or of the ELF field, or the overall intensity $I_S + I_{ELF}$.

In figure 5B, when both fields S and ELF are present, it is possible to modulate not only their intensity or intensity amplitude, but also their ratio I_S/I_{ELF}. For example, different ratios 1; 1.5; 2; etc. can be used for time intervals T_1, T_2; T_3; etc.

Also the frequency can be modulated as shown in figure 5C. The frequency may also be modulated in two or

more following intervals T_1, T_2, wherein the same intensity I_{1-2} is applied.

Starting from the basic examples of figures 5A-5C a sequence of modulated S, ELF, S+ELF fields can be produced that can also be repeated cyclically.

The method according to the invention will now be described in more detail by way of specific examples.

EXAMPLE 1

In this experiment the capability of inducing apoptosis by SELF magnetic field as a function of field intensity and frequency was studied in vitro.

Human colon adenocarcinoma cell line (WiDr) grown in confluent monolayers in T25 flasks was used for the experiment. For each exposure condition 6 flasks containing each about 10 millions cells were used, 3 exposed and 3 shame-exposed (i.e. not exposed).

During the exposure the flasks were held between two coils connected with a circuit providing DC and AC currents up to 100 Hertz. The temperature was continuously monitored and maintained at 37 ± 0,2 °C.

The exposure duration was 20 minutes for each experiment and the SELF field was maintained constant. After 3 hours the cells were treated with May- Grunwald-Giemsa. Apoptosis was assessed by counting the number of apoptotic nuclei per 10 high power fields (HPF) by using an optic microscope.

The amount of induced apoptosis was evaluated by the ratio between the number of apoptotic cells found in the exposure group and the number of apoptotic cells found in the shame-exposed group, that is the group not exposed to the magnetic fields according to the invention.

Table 1 reports the results obtained in different exposure conditions.

TABLE 1

exposure conditions	SELF field composition	frequency (Hz)	field intensity (Static + ELF rms) mT	apoptosis ratio
A	S (static)	-	(0.5 + 0)	1
B	S	-	(1 + 0)	1
C	S	-	(2 + 0)	1.2
D	S	-	(3 + 0)	2
E	S	-	(4 + 0)	2,3
F	S	-	(10 + 0)	2.2
G	S	-	(20 + 0)	2.2
H	S	-	(30 + 0)	2.3
I	ELF	16	(0 + 3)	2.2
L	ELF	33	(0 + 3)	2.2
M	ELF	50	(0 + 3)	2.1
N	ELF	50	(0 + 7)	2,1
O	ELF	66	(0 + 3)	2.2
P	ELF	83	(0 + 3)	2.3
Q	ELF	100	(0 + 3)	2.1
R	S + ELF	50	(4 + 3)	2.1
S	S + ELF	50	50% of time (3 + 1) 50% of time (4,5 + 1,5)	2.2

All the results were statistically highly significant (at the t Student test). From Table 1 we can see that the apoptosis effect appears at 2 mT and doubles starting from 3 mT.

Another important finding is that apoptosis doesn't depend upon SELF field frequency. In other words during the lifetime of the mechanism operating the biological effect (apoptosis) the ELF field is seen as essentially constant. This means that between the two hypothesised mechanism, free- radicals (occurring in a time scale of nano- to microsecond) and ion resonance-like mechanisms, the free radical one is playing the role [[39]Scaiano, 1994, [40]Engstrom, 1997].

EXAMPLE 2

In this experiment the selective effect of SELF magnetic fields was verified exposing three cell lines. Two lines were malignant, human colon adenocarcinoma cells (WiDr) and human breast cancer cells (MCF-7). The normal cell line was human lung fibroblast (MRC-5).

As in the example 1 each cell line was grown in confluent monolayers in T25 flasks. The experimental protocol was the same as in example 1. Six flasks (3 exposed and three shame-exposed) for each cell line were exposed for 20 minutes. Apoptosis was evaluated after 3 hours. The exposure conditions used were the R type of Table 1.

The results are reported in Table 2.

TABLE 2

cell line	apoptosis ratio
WiDr	2.1
MCF-7	1.4
MRC-5	1

As shown in Table 2 only cancer cells reported an apoptosis increment statistically highly significant, whereas the normal cell line didn't. The difference in percentage of apoptosis between the two cancer cell lines was expected due to the two different duplication times. In fact WiDr duplicates faster than MCF-7. The results were evaluated at t Student test.

EXAMPLE 3

In this example nude mice (nu/nu) bearing subcutaneous tumour masses were used to assess the influence of SELF magnetic fields on tumour growth inhibition.

Each mouse was inoculated subcutaneously with 10 million human colon adenocarcinoma cells (WiDr). Two experiments were successively carried out.

In the first experiment, 36 female mice were randomly assigned to 4 experimental groups, each formed by 6 exposed and 3 shame-exposed for a total of 24 animals exposed to 4 different SELF magnetic fields and 12 shame-exposed.

A Static Electric Field up to 6 kV/m was also applied to eventually take advantage of the different electrical behaviour between tumoral and normal tissues [[41]Thornton, 1984; [42]Barsamian, 1987]

In the second experiment 24 female mice were randomly assigned to 2 experimental groups, formed by 12 exposed to the SELF exposure condition which gave the best results among the four exposure conditions used in the previous experiment (exposure condition number 4), and 12 shame-exposed.

All the mice of both experiments were divided into experimental groups after the tumor masses for each animal were palpable.

The animals were exposed for 70 minutes, once a day, for 5 days a week, for 4 weeks. During the exposure each mouse was put in a single box made of Plexiglas held between two coils connected to a circuit providing DC and AC current up to 100 Hz respectively.

Nude mice were kept under specific pathogen free conditions and supplied with "ad libitum" diet. All the tests were conducted in accordance with the protocol issued by N.I.H. (US National Institute of Health) and N.C.I. (US National Cancer Institute).

The tumor masses were measured twice a week and their volume calculated in mm^3 according to the formula:

[(major diameter) x (minor diameter squared)] / 2.

After 4 weeks the animals were sacrificed and autopsied. Tumor masses were extracted, weighed and measured. Portions of tumors were used for different analysis, i.e.

- immunoistochemical: Ki-67 antigen for proliferative index, p-53 antigen for the expression of p-53 gene;

- hystopathological: hematossilina-eosin staining for the assessment of number of mitosis;
- ultrastructural: electron microscopy;
- nucleic acid hybridisation: Tunel method for apoptosis evaluation.

In addition, the following organs were extracted from each animal for histologic examination to assess the treatment toxicity: brain, heart, kidneys, liver, lungs, axillary and inguinal limphonodes, mediastinal limphonodes, ovaries, skin, spleen, bone marrow, subcutaneous tissue (site of tumoral cell line implantation) as well as blood tests.

The obtained results are reported in Table 3 for the first experiment and in Table 4 for the second.

TABLE 3

exposure conditions	1	2	3	4	shame-exposed
exposure duration (min)	70	70	70	70	-
time averaged field intensity (Static + ELF rms) in mT	3	3	4	6	-
field variation in mT (min-max) Static; [min-max] ELF	(4-6) [2-2]	(1.5-4) [1-1]	(2-5) [1.5-3.5]	(2-5) [1.5-3.5]	-
constant field time duration (min-max) in minutes	(5-15)	(5-20)	(5-15)	(5-20)	-
time % with co-presence of Static and ELF fields	0%	50%	50%	100%	-
S/ELF ratio (min-max)	-	(0,5-5)	(0,5-5)	(0,5-5)	-
time % with Static field alone	50%	50%	50%	0%	-
number of mice	6	6	6	6	12
extracted tumor mass volume (mm^3)	1323 ± 304	1450 ± 288	920 ± 540	650 ± 205	1492 ± 559
extract tumor mass weight (g)	1.54 ± 0.22	1.6 ± 0.39	0.98 ± 0.56	0.96 ± 0.25	1.6 ± 0.5
number of apoptotic cells per 10 HPF	98 ± 23	115 ± 20	129 ± 25	129 ± 26	40 ± 17
p53 expression per 10 HPF	35.1 ± 0.11	43.8 ± 0.16	38.2 ± 0.06	28.7 ± 0.14	73.2 ± 0.14

TABLE 4

exposure conditions	4 (see tab. 3)	shame exposed
number of mice	12	12
extracted tumor mass volume	1139 ± 509 cm^3	1914 ± 793 cm^3
extracted tumor mass weight	1.4 ± 0.7 g	2.1 ± 0.6 g
apoptosis (assessed in 50% of mice only)	72.5 ± 9.3	37.0 ± 7.4
p53	35.6± 6.7	78.1±16.7
proliferative index	0.34 ± 0.08	0.45 ± 0.07
mitosis	24.1 ± 10.9	47.7 ± 10.1

The data reported in tables 3 and 4 show that SELF fields have an inhibitory tumor growth effect in vivo. This effect, found in both experiments, was statistically highly significant (in the first experiment, mostly for the exposure condition 4) at the Dunnet and t Student tests respectively.

At the histologic examination of 12 organs for each animal for all groups no differences were found between exposed and shame-exposed mice. No differences were also found in the blood tests. These findings prove the absence of toxicity related to the SELF fields treatment.

The ultrastructural analysis by electron microscope showed in the tumor cells of exposed animals many cellular alterations: presence of apoptotic bodies and condensed chromatin near the nuclear membrane characteristic of apoptotic events.

In addition a consistent result is represented by morphological modifications, increase of number and dimensions of mitochondria as well as number of nucleoli, presence of many vacuoles inside the cytoplasm. Non neoplastic cells (i.e. epithelial and stromal cells) showed no differences between exposed and shame-exposed animals in agreement with the absence of toxicity found in 12 normal organs examined in each animal.

The increment in apoptosis as well as the decrement in p53 gene expression found in exposed mice tumors (see tables 3 and 4) are statistically highly significant (t Student test)

Results reported in Table 3 and 4 are in agreement with those obtained in vitro and shown in Tables 1 and 2.

The effect induced by the SELF magnetic fields on p53 expression enforces the apoptosis results and is in agreement with the hypothesised biophysical mechanism (i.e. free radical recombination) by which the SELF fields have an anti-tumor effect through formation of reactive oxygen species and the degradation of mithocondrial components.

EXAMPLE 4

In this experiment nude mice (nu/nu) previously subcutaneous inoculated with 10 million human colon adenocarcinoma cells (WiDr) were exposed to study the animal survival.

After the cell inoculation 2 groups of mice were randomly formed respectively of 16 animals exposed and 17 shame-exposed. The mice of the former group were exposed 70 minutes once a day, for 5 days a week, for their entire life beginning after 24 hours after the tumor inoculation.

The exposure conditions were the same of the experiment the results which are reported in Table 4.

As in the previous example, the mice were maintained under specific pathogen free condition supplied with "ad libitum" diet. All the tests were conducted in accordance with protocol issued by N.I.H. and N.C.I.

The antitumor effectiveness of the treatment was evaluated by using the N.C.I. formula: ratio between exposed and shame-exposed animals of the average animal life span. This average was evaluated summing for each experimental group the time of survival divided by the number of animals.

The effectiveness is obtained when the N.C.I. formula gives as result an index equal or greater than 1.25.

Table 5 reports for each experimental group, the number of living animals at different times (days) from the beginning of experiment.

TABLE 5

living mice exposed/ shame-exp. (days)	16/16 (48)	16/15 (73)	15/14 (76)	14/14 (84)	13/14 (87)	12/14 (88)
living mice exposed/ shame-exp. (days)	12/13 (97)	12/12 (107)	10/12 (109)	10/10 (114)	10/9 (115)	9/8 (125)
living mice exposed/ shame-exp. (days)	9/7 (149)	8/6 (153)	8/5 (155)	8/4 (157)	7/4 (163)	7/3 (173)
living mice exposed/ shame-exp. (days)	6/3 (183)	6/2 (192)	6/0 (194)	5/0 (195)	4/0 (203)	3/0 (257)
living mice exposed/ shame-exp. (days)	2/0 (276)	1/0 (323)	0*/0 *sacrificed (326)			

The N.C.I. formula applied to the results reported in Table 5 gives an index equal to 1.31, that is greater than 1.25 . After 194 days 6 exposed mice were alive whereas all shame exposed mice were dead.

The foregoing description of specific embodiments will so fully reveal the invention according to the conceptual point of view, so that others, by applying current knowledge, will be able to modify and/or adapt for various applications such embodiments without further research and without departing from the invention, and it is therefore to be understood that such adaptations and modifications will have to be considered as equivalent to the specific embodiments. The means and the materials to realise the different functions described herein could have a different nature without, for this reason, departing from the field of the invention. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation.

REFERENCES

[1] Blank M (1993):"Electricity and Magnetism in Biology and Medicine". The First World Congress for Electricity and Magnetism in Biology and Medicine, Orlando, Florida.

[2] Liboff AR, Williams T Jr, Strong DM and Wistar R. Jr. (1984):"Time-Varying Magnetic Fields: Effect on DNA Synthesis". Science, Vol. 223, pp 818-820.

[3] Tofani S, Ferrara A, Anglesio L, Gilli G (1995):"Evidence for genotoxic effects of resonant ELF magnetic fields". Bioelectrochemistry and Bioenergetics 36, pp 9-13.

[4] Goodman R, Shirley-Henderson A (1991): "Transcription and Translation in Cells exposed to Extremely Low Frequency Electromagnetic Fields" Bioelectrochem. Bioenerg. 25, pp. 335-355.

[5] Phillips jl, Haggren w, Thomas WJ, Ishida-Jones T and Adey WR (1992):"Magnetic field-induced changes in specific gene transcription". Biochimica et Biophysica Acta 1132, pp 140-144.

[6] Liboff AR (1985): Cyclotron resonance in membrane transport. In Chiabrera A, Nicolini C., Schwan HP (eds): "Interactions Between Electromagnetic Fields and Cells". New York: Plenum Press, pp 281-296.

[7] Chiabrera A., Grattarola M., Viviani R. (1984): "Interaction between electromagnetic fields and cells: Microelectrophoretic effect on ligands and surface receptors". Bioelectromagnetics 5, pp173-191.

[8] Lednev VV (1991): "Possible mechanism for the influence of weak magnetic fields on biological systems". Bioelectromagnetics 12, pp 71-75.

[9] Blanchard JP, Blackman CF (1994):"Clarification and application of an ion parametric resonance model for magnetic field interactions with biological systems. Bioelectromagnetics 15, pp217-238.

[10] Preston GA, Barrett JC, Biermann JA and Murphy Elizabeth (1997): "Effects of Alterations in Calcium Homeostasis on Apoptosis during Neoplastic Progression", Cancer Research 57, pp. 537-542.

[11] Trump BF, Berezesky IK, Chang SH and Phelps PC (1997):"The Pathways of Cell Death: Oncosis, Apoptosis, and Necrosis". Toxicologic Pathology Vol. 25, n. 1, pp.82-87.

[12] Grundler W, Kaiser F, Keilmann F, Walleczek J (1992): "Mechanisms of electromagnetic interaction with cellular

systems". Naturwissenschaften 79, pp. 551-559.

[13] Polk C (1992):"Dosimetry of extremely-low-frequency magnetic fields". Bioelectromagnetics Suppl 1, pp. 209-235

[14] Walleczek J, Budinger TF (1992): "Pulsed magnetic field effects on calcium signalling in lymphocytes: Dependence on cell status and field intensity". FEBS Lett 314, pp 351-355.

[15] Adey WR (1993):Electromagnetics in biology and medicine. In Matsumoto H (ed): "Modern Radio Science", New York: Oxford University Press, pp 227-245.

[16] Steiner UE and Ulrich T (1989):"Magnetic Field Effects in Chemical Kinetics and Related Phenomena". Chem. Rev. 89, pp. 51-147.

[17] Lander HM (1997):" An essential role for free radicals and derived species in signal transduction". The FASEB Journal 11, pp118-124.

[18] Polyak K, Xia Y, Zweier JL, Kinzier KW and Volgestein B (1997):"A model for p53-induced apoptosis". Nature Vol. 389, pp. 300-305.

[19] '18). Walch, N.S., Calaoagan, J., Murphy, B.J., Knapp, A.M., Sutherland, R.M., Laderoute, K.R. "The redox-sensitive human antioxidant responsive element induces gene expression under low oxygen conditions". Carcinogenesis, 19 (8): 1333-7,1988.

[20] Amirkhosravi, A., Meyer, T., Warnes, G., Amaya, M., Malik, Z., Biggerstaf, J.P., Siddiqui, F.A., Sherman, P., Francis, J.L. Pentoxifylline inhibits hypoxia-induced upregulation of tumor cell tissue factor and vascular endothelial growth factor. Thromb Haemost, 80 (4): 598-602, 1998.

[21] Cadossi R, Bersani F, Cossarizza A, Zucchini P, Emilia G, Torelli G and Claudio Franceschi (1992):"Lymphocytes and low-frequency electromagnetic fields". The FASEB Journal Vol. 6, pp.2667-2674.

[22] Walleczeck J (1996):"Electromagnetic Field Effects on Cellular Signal Transduction and Free Radical Mechanisms". Abstract Book XXVth General Assembly of the International Union of Radio Science-Lille-France, p. 547.

[23] Binggeli R, Weinstein RC. Membrane potentials and sodium channels: hypotheses for growth regulation and cancer formation based on changes in sodium channels and gap junctions. Theor Biol 1986: 123:377-401.

[24] Marino AA, Iliev IG, Schwalke MA, Gonzales E, Marler KC, Flanagan CA. Association between cell membrane potential and breast cancer Tumour Biol. 1994: 15:82-89.

[25] Davies RJ, Weidema WF, Sandle GI, Palmer LI, Deschener EE, DeCosse JJ. Sodium transport in a mouse model of colonic cancer. Cancer Res. 1987: 47:4646-50.

[26] Goller DA, Weidema WF, Davies RJ. Transmural electrical potential as an early marker in colon cancer. Arch. Surg. 1986: 121:345-50.

[27] Capko D, Zhuravkov A, Davies RJ. Transepithelial depolarisation in breast cancer. Breast Cancer Res. 1996: Treat. 41:230.

[28] Cuzick J, Holland R., Barth V, Davies R, Faupel M, Fentiman I, Frischbier HJ, LaMarque JL, Merson M, Sacchini V, Vanel D, Veronesi U. Electropotential measurements as a new diagnostic modality for breast cancer. The Lancet 1998: 352:359-363.

[29] Szatrowski TP, Nathan CF. Production of of large amounts of hydrogen peroxide by human tumor cells. Cancer Res. 1991: 51 (3):794-798.

[30] Shulyakovskaya T, Sumegi L, Gal D. In vivo experimental studies on the role of free radicals in photodynamic therapy. I. measurement of the steady state concentration of free radicals in tumor tissues of mice. Biochem. Biophys. Res. Commun. 1993: 195 (2):581-587.

[31] Iwagaki H, Hamazaki K, Matsubara N, Hiramatsu M, Orita K, Mori A..Lipid peroxidation in hepatocellular carcinoma. Acta Med. Okayama 1995: 49 (6):313-315.

[32] Levin VA (1998):"Signal Transduction Directed Therapy: Fact or Fantasy?" Abstract Book (EL 5) of the Eight International Congress on Anti-Cancer Treatment, February 3rd-6th 1998, Paris, France.

[33] Thompson C.B. (1995):"Apoptosis in the pathogenesis and treatment of diseases" Science Vol. 267, p. 1456-1462

[34] Costa JL and Hofmann GA (1987): "Malignancy treatment" U.S. patent 4,665,898.

[35] Narita K, Hanakawa K, Kasahara T, Hisamitsu T, Asano K (1997):"Induction of apoptotic cell death in human leukemic cell line, HL-60, by extremely low frequency electric magnetic fields: analysis of the possible mechanisms in vitro". In vivo 111(4), pp. 329-335.

[36] Raylman RR, Clavo AC, Wahl RL (1996):"Exposure to Strong Static Magnetic Field Slow the Growth of Human Cancer Cells In Vitro". Bioelectromagnetics 17, pp. 358-363.

[37] Haberkorn R, Michel-Beyerle ME. On the mechanism of magnetic field effects in bacterial photosynthesis. Biophysical Journal 1979: 26:489-498.

[38] Lersch W, Michel-Beyerle ME. Magnetic field effects on the recombination of radical ions in reaction centers of photosynthetic bacteria. Chemical Physics 1983: 78:115-126.

[39] Scaiano JC, Mohtat N, Cozens FL, McLean J and Thansandote (1994):"Application of the Radical Pair Mechanism to Free Radicals I Organized Systems: Can the Effects of 60 Hz Be Predicted From Studies Under Static Fields?" Bioelectromagnetics 15, pp.549-554.

[40] Engstrom S (1997):"What is the Time of Magnetic Field Interaction in Biological Systems?". Bioelectromagnetics 18, pp. 244-249.

[41] B.S. Thornton (1984): "Inversion of raman spectra of living cells indicates dielectric structure related to energy control", in Physics Letters, Vol. 106A, pp. 198-202.

[42] S.T. Barsamian (1987): "Dielectric origin of living cells", in Biophysical Aspects of Cancer, Charles University Prague, pp. 152-159

CLAIMS

1. Apparatus for selectively interfering with pathological cells survival processes in vitro and in vivo **characterised in that** it comprises:

- means for generating static magnetic (S) fields crossing a working environment,
- means for generating electromagnetic extremely low frequency (ELF) fields over said working environment in addition to said S fields;
- means for modulating said S fields associated to said means for generating S fields, said means for modulating said S fields setting the intensity of said S fields between 1 and 100 mT according to a predetermined function of intensity versus time;
- means for modulating said ELF fields associated to said means for generating ELF fields, said means for modulating said ELF fields setting said ELF fields according to a predetermined function of amplitude of intensity between 1 and 100 mT and frequency between 1 and 1000 Hz versus time.

2. Apparatus for selectively interfering with pathological cells survival processes in vitro and in vivo **characterised in that** it comprises:

- means for generating static magnetic (S) fields crossing a working environment,
- means for modulating said S fields associated to said generating means, said means for modulating the S fields setting the intensity of said S fields between 1 and 100 mT according to a predetermined function of intensity versus time.

3. Apparatus for selectively interfering with pathological cells survival processes in vitro and in vivo **characterised in that** it comprises:

- means for generating electromagnetic extremely low

frequency (ELF) fields over said working environment;

- means for modulating said ELF fields associated to said means for generating, said means for modulating said ELF fields setting said ELF fields according to a predetermined function of amplitude of intensity between 1 and 100 mT and frequency between 1 and 1000 Hz versus time.

4. Apparatus according to any of claims 1 or 2 wherein said means for modulating said S fields comprises program means that set said intensity following a plurality of predetermined step values I_{s1}, I_{s2}, ...,I_{sn} for corresponding time intervals T_1, T_2, ..., T_n.

5. Apparatus according to any of claims 1 or 3 wherein said means for modulating said ELF fields comprises program means that set said intensity amplitude following a plurality of predetermined step values I_{ELF1}, I_{ELF2}, ..., I_{ELFn} for corresponding time intervals T_1, T_2, ..., T_n.

6. Apparatus according to any of claims 1 or 3 wherein said means for modulating said ELF fields comprises program means that set said frequency following a plurality of predetermined step values f_1, f_2,..., f_n, for corresponding time intervals T_1, T_2, ..., T_n, said step values being comprised between 10 and 100 Hz.

7. Apparatus according to claim 1, wherein said means for modulating said S and ELF fields comprises program means that set an S/ELF ratio according to a plurality of predetermined step values I_{s1}/I_{ELF1}, I_{s2}/I_{ELF2},...,I_{sn}/I_{ELFn}, for corresponding time intervals T_1, T_2, ..., T_n,.

8. Apparatus according to claim 7, wherein said program means set said S and ELF fields according to an overall intensity between 1 and 30 mT and respectively a ratio S/ELF comprised between 0,1 and 10.

9. Apparatus according to claim 7, wherein said program means set said S and ELF fields according to an overall

intensity between 1 and 10 mT and respectively a ratio S/ELF comprised between 0,5 and 5.

10. Apparatus according to claims 4 to 9 wherein said program means set said time intervals between 1 and 40 minutes.

11. Apparatus according to the previous claims wherein at least a portion of said working environment is defined by walls permeable to said fields.

12. Apparatus according to the previous claims, wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil respectively surrounding at least a portion of said working environment, said means for modulating providing to said coils DC and/or AC current respectively.

13. Apparatus according to the claims from 1 to 11, wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil coaxial to each other, said working environment being placed between said first and a second coil and said means for modulating providing to said coils DC and/or AC current respectively.

14. Apparatus according to the previous claims, wherein means are provided for creating through said working environment a static electric field, or a low frequency variable electric field up to 1000 Hz, having intensity up to 20 kV/m.

15. The use of SELF non thermal fields for selectively interfering with pathological cells survival, such as in particular cells affected by cancer, viral infections, autoimmune diseases, neurodegenerative disorders, AIDS, etc., characterised in that said SELF non thermal fields have intensity comprised between 1 and 100 mT, said SELF fields being different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the

presence of S or ELF fields alone, said ELF fields having a field frequency comprised between 1 and 1000 Hz.

16. The use of SELF non thermal fields for biotechnological genes modifications, such as in particular for modification of mutant p53 gene, characterised in that said SELF non thermal fields have intensity comprised between 1 and 100 mT, said SELF fields being different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, said ELF fields having a field frequency comprised between 1 and 1000 Hz.

17. The use of SELF non thermal fields according to claims 15 or 16, wherein chemical substances are used in addition to the SELF fields.

18. The use of SELF non thermal fields according to claims 15 or 16, wherein said different sequences of S and/or ELF fields sequences are set for time intervals T_1, T_2, ..., T_n, and wherein in said time intervals the intensity of said S and/or ELF fields are set at steady values I_{S1}, I_{S2}, ..., I_{Sn}; I_{ELF1}, I_{ELF2}, ..., I_{ELFn}, I_{S1}/I_{ELF1}, I_{S2}/I_{ELF2}, ..., I_{Sn}/I_{ELFn}, respectively.

19. The use of SELF non thermal fields according to claims 15 or 16, wherein said S and ELF fields are set at an overall intensity between 1 and 30 mT with respectively a ratio S/ELF comprised between 0,1 and 10.

20. The use of SELF non thermal fields according to claims 15 or 16, wherein said S and ELF fields are set at an overall intensity between 1 and 10 mT with respectively a ratio S/ELF comprised between 0,5 and 2,5.

Fig. 1

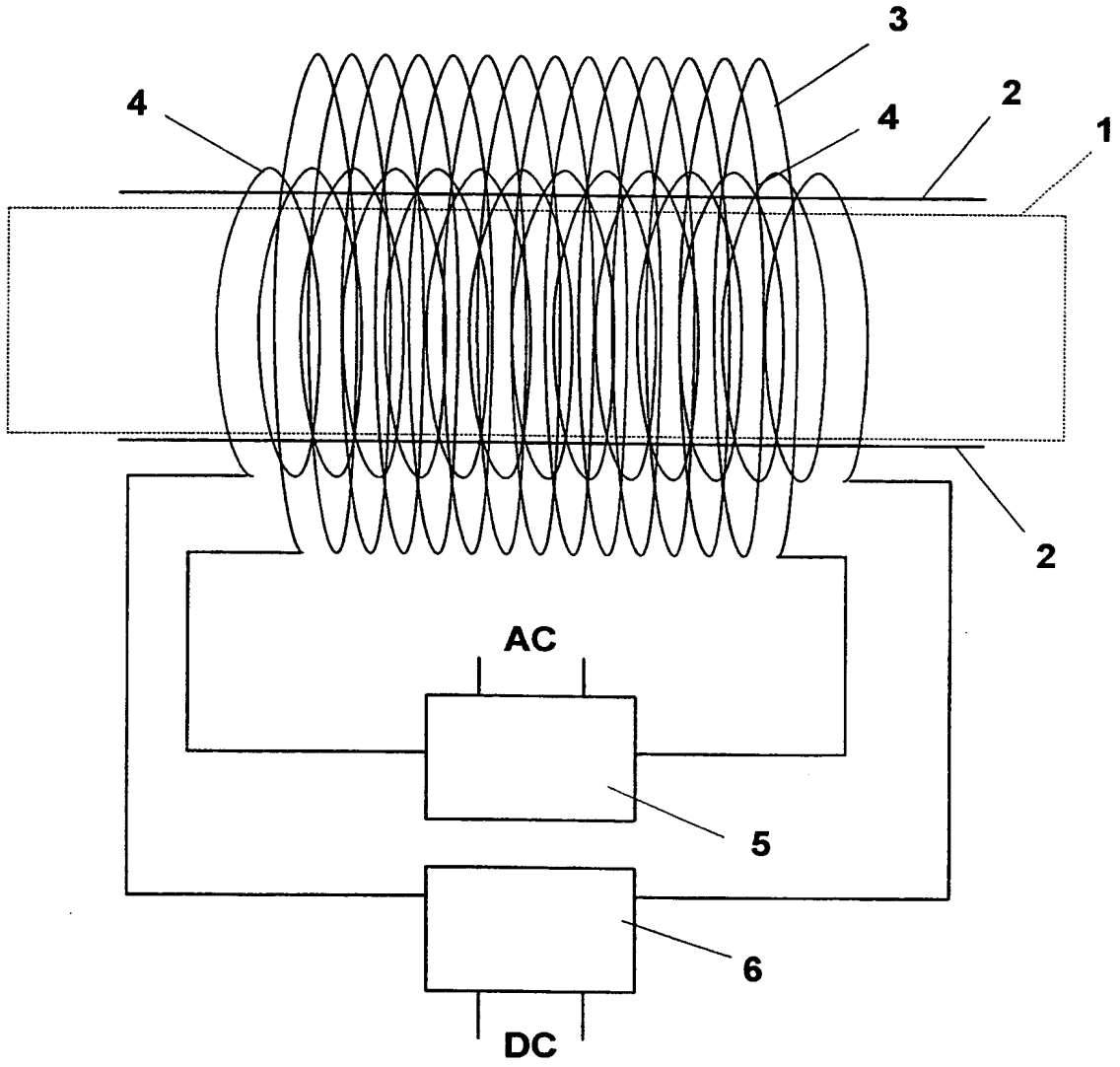


Fig. 2

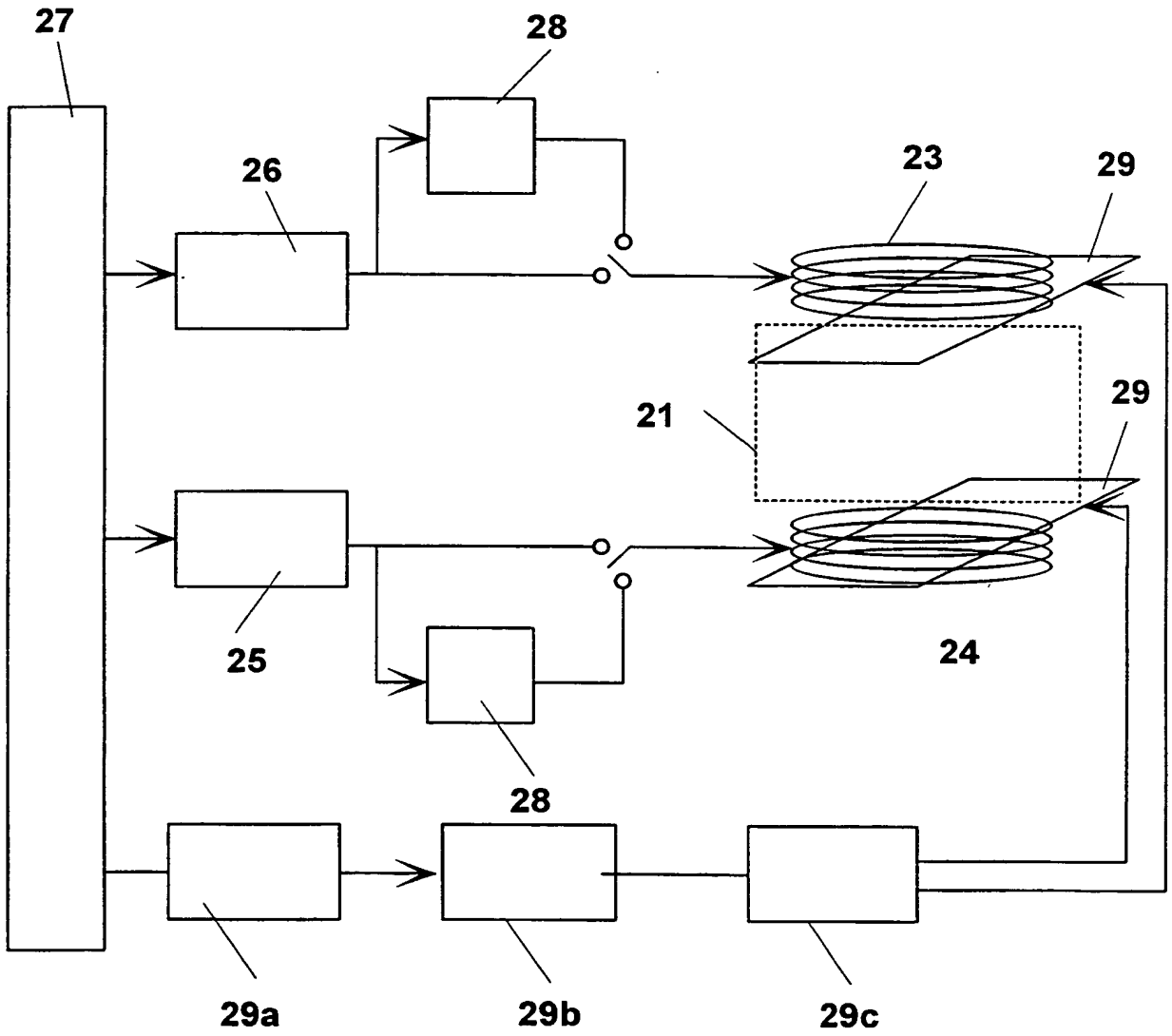
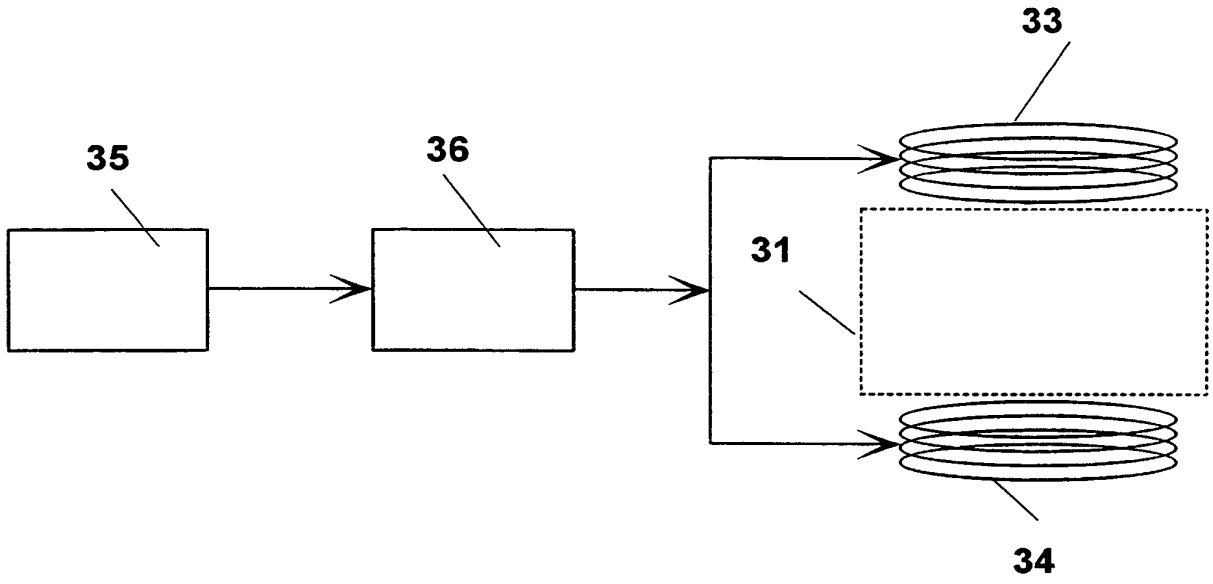


Fig. 3



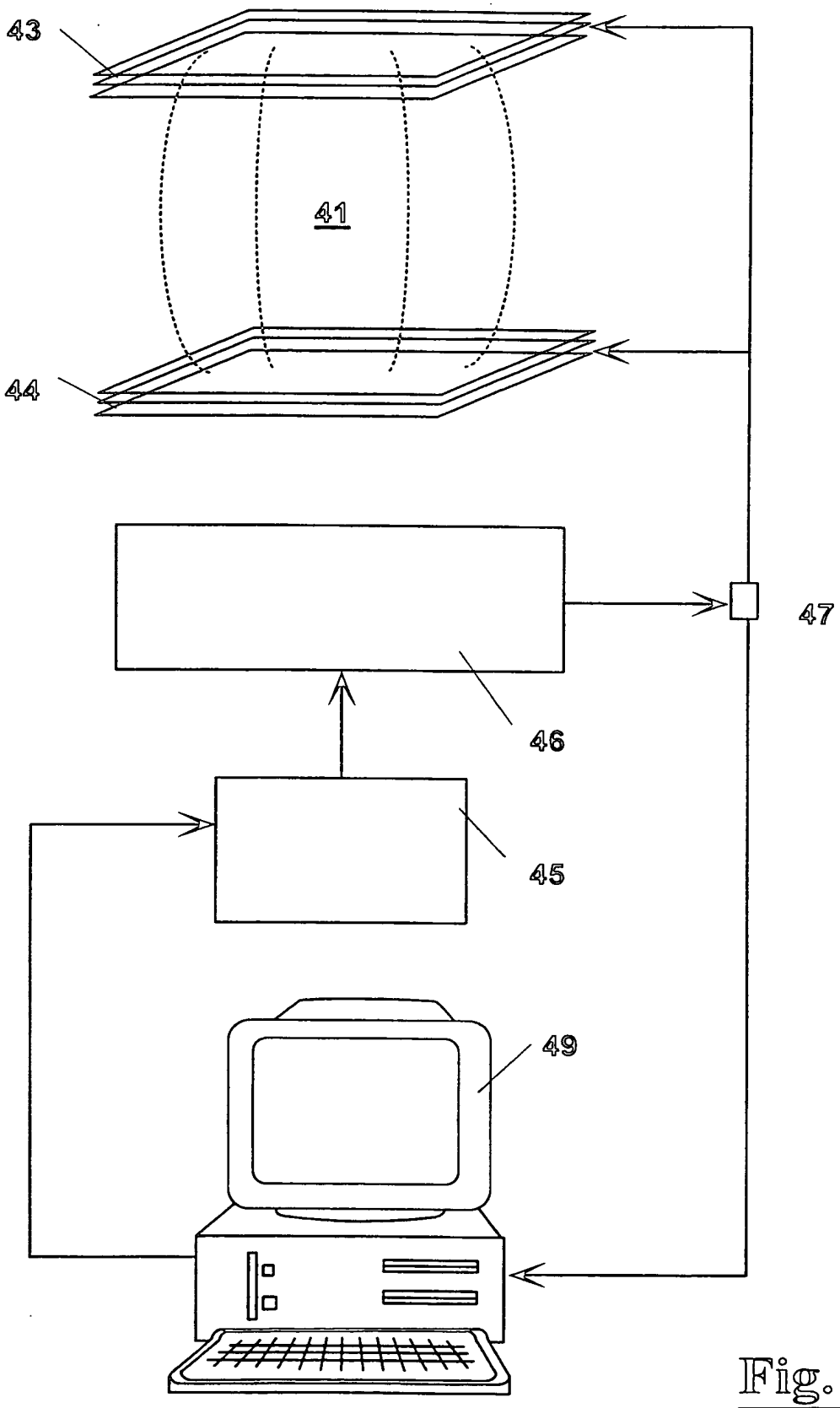


Fig. 4

Fig. 5A

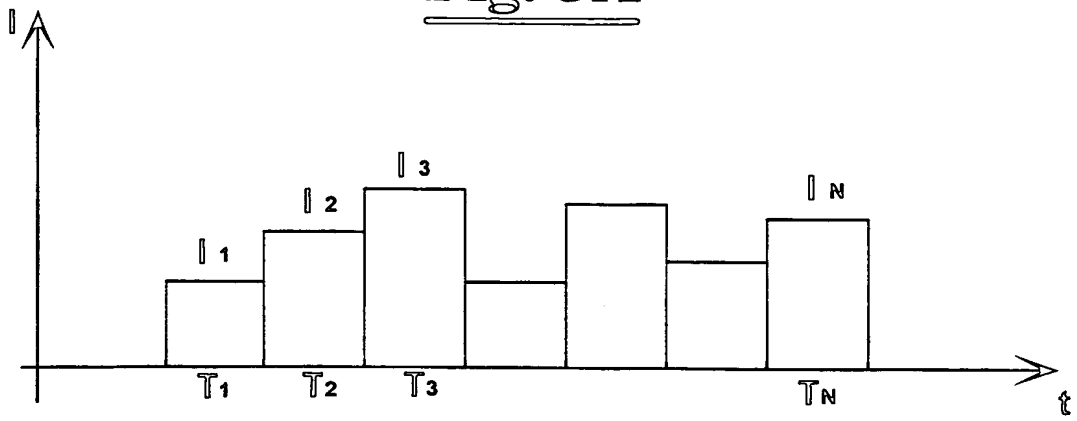


Fig. 5B

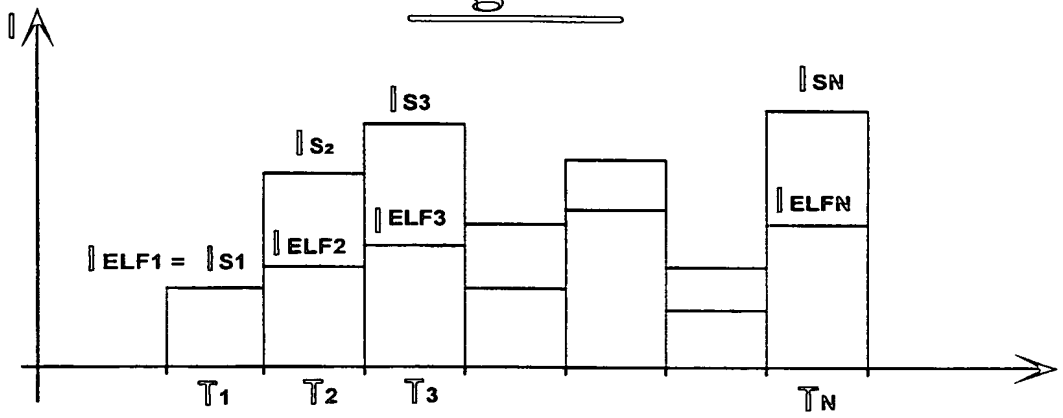
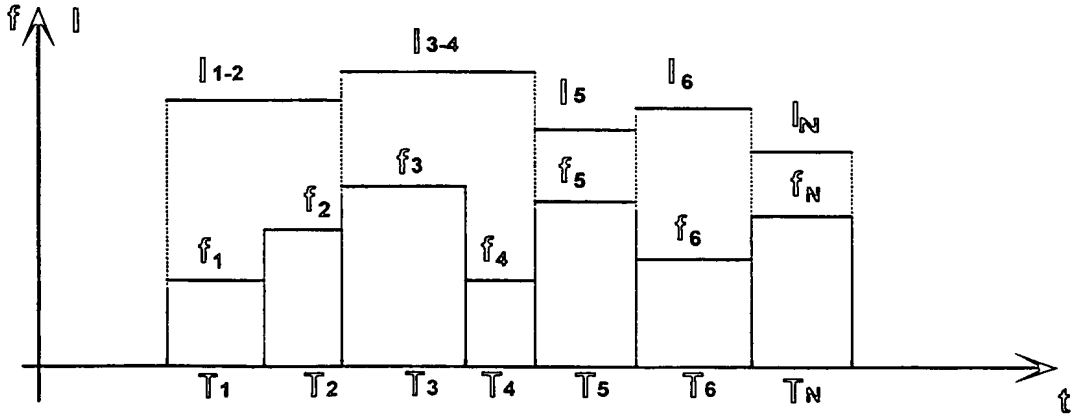


Fig. 5C



INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/04385

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61N2/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	US 5 156 587 A (MONTONE LIBER J) 20 October 1992 (1992-10-20) column 1, line 10-21 column 2, line 58-68 column 5, line 37 -column 6, line 16 column 10, line 13-20 ---	3,12,13, 15 1,5,10, 16
X A	DE 39 11 393 A (KRAUS WERNER) 11 October 1990 (1990-10-11) the whole document ---	3,11-13, 15 1,2,5, 10,16
X A	DE 41 22 380 A (KRAUS WERNER) 7 January 1993 (1993-01-07) column 2, line 68 -column 3, line 22 --- -/--	3,15 1,2,16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
29 September 1999	06/10/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Grossmann, C.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropnate, of the relevant passages	Relevant to claim No.
A	DE 40 36 770 A (LIFE RESONANCES, INC.) 16 May 1991 (1991-05-16) page 3, line 17 -page 4, line 28	1-3,8,9, 12,13, 15,16, 19,20
A	WO 96 39493 A (U S ENVIRONMENTAL PROTECTION A ;BLACKMAN CARL F (US); BLANCHARD JA) 12 December 1996 (1996-12-12) page 27, line 13 -page 30, line 30	1-3,8,9, 12,13, 15-17, 19,20
A	TOFANI S;, FERRARA A, ANGLESIO L, GILLI G: "evidence for genotoxic effects of resonant elf magnetic fields" BIOELECTROCHEMISTRY AND BIOENERGETICS, no. 36, 1995, page 9-13 XP002084038 cited in the application the whole document	1-3,8,9, 12,13, 15,16, 19,20
A	US 5 691 324 A (SANDYK REUVEN) 25 November 1997 (1997-11-25) column 8, line 36 -column 9, line 27 column 11, line 6-28 column 16, line 9-22 column 17, line 52 -column 18, line 33	1-5,9, 12,15-19
A	WO 97 04830 A (GRAY JAMES R) 13 February 1997 (1997-02-13) page 10, line 18-21 page 15, line 8-16 page 44, line 19 -page 47, line 12 page 52, line 15 -page 54, line 6	2,4,11, 14,15, 17,18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/04385

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5156587 A	20-10-1992	NONE	
DE 3911393 A	11-10-1990	NONE	
DE 4122380 A	07-01-1993	NONE	
DE 4036770 A	16-05-1991	US 5045050 A	03-09-1991
		AU 6592390 A	23-05-1991
		CA 2029398 A	16-05-1991
		US 5437600 A	01-08-1995
		US 5211622 A	18-05-1993
		US 5183456 A	02-02-1993
WO 9639493 A	12-12-1996	AU 3596895 A	24-12-1996
		US 5919679 A	06-07-1999
US 5691324 A	25-11-1997	US 5470846 A	28-11-1995
		WO 9913884 A	25-03-1999
		US 5885976 A	23-03-1999
		US 5691325 A	25-11-1997
		AU 4486797 A	05-04-1995
WO 9704830 A	13-02-1997	AU 6639596 A	26-02-1997

INTERNATIONAL SEARCH REPORT

[redacted]ation on patent family members

[redacted]rnational Application No
[redacted]T/EP 99/04385

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5156587 A	20-10-1992	NONE	
DE 3911393 A	11-10-1990	NONE	
DE 4122380 A	07-01-1993	NONE	
DE 4036770 A	16-05-1991	US 5045050 A	03-09-1991
		AU 6592390 A	23-05-1991
		CA 2029398 A	16-05-1991
		US 5437600 A	01-08-1995
		US 5211622 A	18-05-1993
		US 5183456 A	02-02-1993
WO 9639493 A	12-12-1996	AU 3596895 A	24-12-1996
		US 5919679 A	06-07-1999
US 5691324 A	25-11-1997	US 5470846 A	28-11-1995
		WO 9913884 A	25-03-1999
		US 5885976 A	23-03-1999
		US 5691325 A	25-11-1997
		AU 4486797 A	05-04-1995
WO 9704830 A	13-02-1997	AU 6639596 A	26-02-1997

CLAIMS

1. Apparatus for selectively interfering with pathological cells survival processes in vitro and in vivo comprising:

- means for generating static magnetic (S) fields crossing a working environment,

- means for generating electromagnetic extremely low frequency (ELF) fields over said working environment in addition to said S fields;

characterised in that it further comprises:

- means for modulating said S fields associated to said means for generating S fields, said means for modulating said S fields setting the intensity of said S fields between 1 and 100 mT according to a predetermined function of intensity versus time;

- means for modulating said ELF fields associated to said means for generating ELF fields, said means for modulating said ELF fields setting said ELF fields according to a predetermined function of amplitude of intensity between 1 and 100 mT and frequency between 1 and 1000 Hz versus time.

2. Apparatus for selectively interfering with pathological cells survival processes in vitro and in vivo comprising:

- means for generating static magnetic (S) fields crossing a working environment,

characterised in that it further comprises

- means for modulating said S fields associated to said generating means, said means for modulating the S fields

setting the intensity of said S fields between 1 and 100 mT according to a predetermined function of intensity versus time.

3. Apparatus for selectively interfering with pathological cells survival processes in vitro and in vivo

characterised in that it further comprises

- means for generating electromagnetic extremely low frequency (ELF) fields over said working environment;
- means for modulating said ELF fields associated to said means for generating, said means for modulating said ELF fields setting said ELF fields according to a predetermined function of amplitude of intensity between 1 and 100 mT and frequency between 1 and 1000 Hz versus time.

4. Apparatus according to any of claims 1 or 2 wherein said means for modulating said S fields comprises program means that set said intensity following a plurality of predetermined step values I_{s1}, I_{s2}, ▒, I_{sn} for corresponding time intervals T_1, T_2, ▒, T_n.

5. Apparatus according to any of claims 1 or 3 wherein said means for modulating said ELF fields comprises program means that set said intensity amplitude following a plurality of predetermined step values I_{ELF1}, I_{ELF2}, ▒, I_{ELFn} for corresponding time intervals T_1, T_2, ▒, T_n.

6. Apparatus according to any of claims 1 or 3 wherein said means for modulating said ELF fields comprises program means that set said frequency following a plurality of predetermined step values f_1, f_2, ▒, f_n, for corresponding time intervals T_1, T_2, ▒, T_n, said step values being comprised between 10 and 100 Hz.

7. Apparatus according to claim 1, wherein said means for modulating said S and ELF fields comprises program means that set an S/ELF ratio according to a plurality of predetermined step values I_{s1}/I_{ELF1}, I_{s2}/I_{ELF2}, ▒, I_{sn}/I_{ELFn}, for corresponding time intervals T_1, T_2, ▒, T_n, .

8. Apparatus according to claim 7, wherein said program means set said S and ELF fields according to an overall intensity between 1 and 30 mT and respectively a ratio S/ELF comprised between 0,1 and 10.

9. Apparatus according to claim 7, wherein said program means set said S and ELF fields according to an overall intensity between 1 and 10 mT and respectively a ratio S/ELF comprised between 0,5 and 5.

10. Apparatus according to claims 4 to 9 wherein said program means set said time intervals between 1 and 40 minutes.

11. Apparatus according to the previous claims wherein at least a portion of said working environment is defined by walls permeable to said fields.

12. Apparatus according to the previous claims, wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil respectively surrounding at least a portion of said working environment, said means for modulating providing to said coils DC and/or AC current respectively.

13. Apparatus according to the claims from 1 to 11, wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil coaxial to each other, said working environment being placed between said first and a second coil and said means for modulating providing to said coils DC and/or AC current respectively.

14. Apparatus according to the previous claims, wherein means are provided for creating through said working environment a static electric field, or a low frequency variable electric field up to 1000 Hz, having intensity up to 20 kV/m.

15. The use of SELF non thermal fields for selectively interfering with pathological cells survival, such as in particular cells affected by cancer, viral infections, autoimmune diseases, neurodegenerative disorders, AIDS,

etc., characterised in that said SELF non thermal fields have intensity comprised between 1 and 100 mT, said SELF fields being different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, said ELF fields having a field frequency comprised between 1 and 1000 Hz.

16. The use of SELF non thermal fields for biotechnological genes modifications, such as in particular for modification of mutant p53 gene, characterised in that said SELF non thermal fields have intensity comprised between 1 and 100 mT, said SELF fields being different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, said ELF fields having a field frequency comprised between 1 and 1000 Hz.

17. The use of SELF non thermal fields according to claims 15 or 16, wherein chemical substances are used in addition to the SELF fields.

18. The use of SELF non thermal fields according to claims 15 or 16, wherein said different sequences of S and/or ELF fields sequences are set for time intervals T_1, T_2, ▒, T_n, and wherein in said time intervals the intensity of said S and/or ELF fields are set at steady values I_{s1}, I_{s2}, ▒, I_{sn}; I_{ELF1}, I_{ELF2}, ▒, I_{ELFn}, I_{s1}/I_{ELF1}, I_{s2}/I_{ELF2}, ▒, I_{sn}/I_{ELFn}, respectively.

19. The use of SELF non thermal fields according to claims 15 or 16, wherein said S and ELF fields are set at an overall intensity between 1 and 30 mT with respectively a ratio S/ELF comprised between 0,1 and 10.

20. The use of SELF non thermal fields according to claims 15 or 16, wherein said S and ELF fields are set at an overall intensity between 1 and 10 mT with respectively a ratio S/ELF comprised between 0,5 and 2,5.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
B30/0020		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/04385	23/06/1999	24/06/1998
International Patent Classification (IPC) or national classification and IPC A61N2/02		
Applicant TOFANI, SANTI		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☐ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/01/2000	07.07.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Stern, M Telephone No. +49 89 2399 2239

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/04385

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-23 as originally filed

Claims, No.:

1-20 as originally filed

Drawings, sheets:

1/5-5/5 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-20.

because:

☒ the said international application, or the said claims Nos. 15-20 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 1-14 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

Regarding Section III:

1. The subject-matter of method claims 15-20 is directed at methods for interfering, in particular, with in vivo cells, such as cells affected by autoimmune diseases or cancer. Consequently, the claimed methods pertain to methods of treatment of the human or animal body (Article 34(4)(a)(i) and Rule 67.1(iv) PCT).

2. Concerning apparatus claims 1-14, it is at present not clear what contribution the present invention sets out to make, whereby the definition of the invention lacks clarity in the sense of Art. 6 PCT. The reasons are given hereinafter.

2.1 The invention is defined in three alternative independent claims, defining in essence:

(a) modulated electromagnetic extremely low frequency (ELF) fields (independent claim 3);
(b) modulated static (S) magnetic fields (independent claim 2); and
(c) modulated S and ELF fields (independent claim 1)

2.2 The description of the invention departs from the knowledge disclosed in particular in document **DE-A-4 122 380 (D1)**. This document already discloses modulated ELF fields as defined in independent claim 3 (cf D1, column 3, lines 10-15); see point 2.4 below. It is therefore not clear which are the essential aspects of the present invention. In particular, these cannot consist in the introduction of a modulation function to magnetic fields, the only common feature to (a), (b) and (c). As a consequence, the three independent claims do not clearly specify the essential novel and inventive feature of the invention by which a technical problem is to be solved (Art. 6 PCT taken in combination with Rule 6.3(a), (b) PCT).

2.3 Moreover, it seems that the present invention does not show in a clear and convincing way that any technical problem has in fact been solved (Rule 6.3(b) PCT). The statistical significances mentioned in the examples given in the application have been established with regard to already known parameters, but not with respect to the (non-recited) essential feature mentioned above. In the absence of such convincing evidence, an alleged essential feature to solve an

alleged problem will most likely have to be deemed as being merely a speculative parameter which does not produce any technical effect (other than creating a different apparatus from the prior art).

2.4 Furthermore, the subject-matter of claims 1 and 3-14 lacks clarity (Art. 6 PCT) since the concept of an "extremely low frequency" field is ambiguous, leaving the reader in doubt as to the exact range of frequencies (cf PCT-Guidelines, III, 4.5). The range of frequencies (given, eg, on page 1, lines 28-29) will have to be included in the claim.

3. The aforementioned objections of lack of clarity preclude any meaningful assessment of novelty and inventive step. Nevertheless, the following points are added for the applicant's information:

3.1 It is noted that, apart from the lack of clarity mentioned above, the apparatus recited in independent claim 2 is anticipated by document **WO-A- 97/04830 (D2)** (see in D2, page 52, lines 4-24; Fig. 11-13).

3.2 It is also noted that D1 discloses modulated ELF fields and pulsed S fields (the latter of which contain, for all practical purposes, a modulation equivalent to that recited in claim 2). Hence, apart from the lack of clarity mentioned above, the definition of claim 1 would have resulted obvious from D1 in view of the fact that the combination of S and ELF fields was already known in the prior art from the publication by the inventor, mentioned under [3] on page 2, line 8 of the application, that is,

D3: Tofani et al: "Evidence for genotoxic effects of resonant elf magnetic fields"; Bioelectrochemistry and Bioenergetics; No. 36, 1995; pages 9-13.

See in D3, page 11, paragraph 3.

3.3 To meet the requirements of Rule 6.3(b) PCT the independent claim should have been properly cast in the two part form, with those features which in combination are known from D1 being placed in the preamble.

3.4 Reference signs in parentheses should have been inserted in the claims to increase their intelligibility, Rule 6.2(b) PCT. This applies to both the preamble and characterising portion.

15

PATENT COOPERATION TREATY

PCT

(New Version)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
B30/0020		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/04385	23/06/1999	24/06/1998
International Patent Classification (IPC) or national classification and IPC A61N2/02		
Applicant TOFANI, SANTI		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☐ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/01/2000	29.09.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Stern, M Telephone No. +49 89 2399 2239

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/04385

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-23 as originally filed

Claims, No.:

1-20 with telefax of 06/07/2000

Drawings, sheets:

1/5-5/5 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-20.

because:

☒ the said international application, or the said claims Nos. 15-20 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 1-14 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

Regarding Section I:

For an International Preliminary Examination Report, no auxiliary requests can be considered. Hence, the present report is based on claims 1-20 as filed on 06.07.00 under the heading "main request". (These claims are in substance identical to original claims 1-20.)

Regarding Section III:

1. The subject-matter of method claims 15-20 is directed at methods for interfering, in particular, with in vivo cells, such as cells affected by autoimmune diseases or cancer. Consequently, the claimed methods pertain to methods of treatment of the human or animal body (Article 34(4)(a)(i) and Rule 67.1(iv) PCT).

2. Concerning apparatus claims 1-14, it is at present not clear what contribution the present invention sets out to make, whereby the definition of the invention lacks clarity in the sense of Art. 6 PCT. The reasons are given hereinafter.

2.1 The invention is defined in three alternative independent claims, defining in essence:

(a)modulated electromagnetic extremely low frequency (ELF) fields (independent claim 3);
(b) modulated static (S) magnetic fields (independent claim 2); and
(c) modulated S and ELF fields (independent claim 1)

2.2 The description of the invention departs from the knowledge disclosed in particular in document **DE-A-4 122 380 (D1)**. This document already discloses modulated ELF fields as defined in independent claim 3 (cf D1, column 3, lines 10-15); see point 2.4 below. It is therefore not clear which are the essential aspects of the present invention. In particular, these cannot consist in the introduction of a modulation function to magnetic fields, the only common feature to (a), (b) and (c). As a consequence, the three independent claims do not clearly specify the essential novel and inventive feature of the invention by which a technical problem

is to be solved (Art. 6 PCT taken in combination with Rule 6.3(a), (b) PCT).

2.3 Moreover, it seems that the present invention does not show in a clear and convincing way that any technical problem has in fact been solved (Rule 6.3(b) PCT). The statistical significances mentioned in the examples given in the application have been established with regard to already known parameters, but not with respect to the (non-recited) essential feature mentioned above. In the absence of such convincing evidence, an alleged essential feature to solve an alleged problem will most likely have to be deemed as being merely a speculative parameter which does not produce any technical effect (other than creating a different apparatus from the prior art).

2.4 Furthermore, the subject-matter of claims 1 and 3-14 lacks clarity (Art. 6 PCT) since the concept of an "extremely low frequency" field is ambiguous, leaving the reader in doubt as to the exact range of frequencies (cf PCT-Guidelines, III, 4.5). The range of frequencies (given, eg, on page 1, lines 28-29) will have to be included in the claim.

3. The aforementioned objections of lack of clarity preclude any meaningful assessment of novelty and inventive step. Nevertheless, the following points are added for the applicant's information:

3.1 It is noted that, apart from the lack of clarity mentioned above, the apparatus recited in independent claim 2 is anticipated by document **WO-A- 97/04830 (D2)** (see in D2, page 52, lines 4-24; Fig. 11-13).

3.2 It is also noted that D1 discloses modulated ELF fields and pulsed S fields (the latter of which contain, for all practical purposes, a modulation equivalent to that recited in claim 2). Hence, apart from the lack of clarity mentioned above, the definition of claim 1 would have resulted obvious from D1 in view of the fact that the combination of S and ELF fields was already known in the prior art from the publication by the inventor, mentioned under [3] on page 2, line 8 of the application, that is,

D3: Tofani et al: "Evidence for genotoxic effects of resonant elf magnetic fields"; Bioelectrochemistry and Bioenergetics; No. 36, 1995; pages 9-13.

See in D3, page 11, paragraph 3.

3.3 To meet the requirements of Rule 6.3(b) PCT the independent claim should have been properly cast in the two part form, with those features which in combination are known from D1 being placed in the preamble.

3.4 Reference signs in parentheses should have been inserted in the claims to increase their intelligibility, Rule 6.2(b) PCT. This applies to both the preamble and characterising portion.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference		See Notification of Transmittal of International
[illegible]	[illegible]	[illegible]
International application No	International filing date (day/month/year)	Priority date (day/month/year)
[illegible]	23/06/1999	24/06/1998
International Patent Classification (IPC) or national classification and IPC		
A01N2/02		
Applicant		
[illegible]		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 5 sheet(s).

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☐ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/01/2000	29.09.2000
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Storm, M Telephone No. +49 89 2399 2239

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/04365

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*).

Description, pages:

1-23 as originally filed

Claims, No.:

1-20 with telefax of 06/07/2000

Drawings, sheets:

1/5-5/5 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-20.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/04422

because:

☒ the said international application, or the said claims Nos. 15-20 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 1-14 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

Regarding Section I:

For an International Preliminary Examination Report, no auxiliary requests can be considered. Hence, the present report is based on claims 1-20 as filed on 06.07.00 under the heading "main request". (These claims are in substance identical to original claims 1-20.)

Regarding Section III:

1. The subject-matter of method claims 15-20 is directed at methods for interfering, in particular, with in vivo cells, such as cells affected by autoimmune diseases or cancer. Consequently, the claimed methods pertain to methods of treatment of the human or animal body (Article 34(4)(a)(i) and Rule 67.1(iv) PCT).

2. Concerning apparatus claims 1-14, it is at present not clear what contribution the present invention sets out to make, whereby the definition of the invention lacks clarity in the sense of Art. 6 PCT. The reasons are given hereinafter.

2.1 The invention is defined in three alternative independent claims, defining in essence:

(a)modulated electromagnetic extremely low frequency (ELF) fields (independent claim 3);
(b) modulated static (S) magnetic fields (independent claim 2); and
(c) modulated S and ELF fields (independent claim 1)

2.2 The description of the invention departs from the knowledge disclosed in particular in document **DE-A-4 122 380 (D1)**. This document already discloses modulated ELF fields as defined in independent claim 3 (cf D1, column 3, lines 10-15); see point 2.4 below. It is therefore not clear which are the essential aspects of the present invention. In particular, these cannot consist in the introduction of a modulation function to magnetic fields, the only common feature to (a), (b) and (c). As a consequence, the three independent claims do not clearly specify the essential novel and inventive feature of the invention by which a technical problem

is to be solved (Art. 6 PCT taken in combination with Rule 6.3(a), (b) PCT).

2.3 Moreover, it seems that the present invention does not show in a clear and convincing way that any technical problem has in fact been solved (Rule 6.3(b) PCT). The statistical significances mentioned in the examples given in the application have been established with regard to already known parameters, but not with respect to the (non-recited) essential feature mentioned above. In the absence of such convincing evidence, an alleged essential feature to solve an alleged problem will most likely have to be deemed as being merely a speculative parameter which does not produce any technical effect (other than creating a different apparatus from the prior art).

2.4 Furthermore, the subject-matter of claims 1 and 3-14 lacks clarity (Art. 6 PCT) since the concept of an "extremely low frequency" field is ambiguous, leaving the reader in doubt as to the exact range of frequencies (cf PCT-Guidelines, III, 4.5). The range of frequencies (given, eg, on page 1, lines 28-29) will have to be included in the claim.

3. The aforementioned objections of lack of clarity preclude any meaningful assessment of novelty and inventive step. Nevertheless, the following points are added for the applicant's information:

3.1 It is noted that, apart from the lack of clarity mentioned above, the apparatus recited in independent claim 2 is anticipated by document **WO-A- 97/04830 (D2)** (see in D2, page 52, lines 4-24; Fig. 11-13).

3.2 It is also noted that D1 discloses modulated ELF fields and pulsed S fields (the latter of which contain, for all practical purposes, a modulation equivalent to that recited in claim 2). Hence, apart from the lack of clarity mentioned above, the definition of claim 1 would have resulted obvious from D1 in view of the fact that the combination of S and ELF fields was already known in the prior art from the publication by the inventor, mentioned under [3] on page 2, line 8 of the application, that is,

D3: Tofani et al: "Evidence for genotoxic effects of resonant elf magnetic fields"; Bioelectrochemistry and Bioenergetics; No. 36, 1995; pages 9-13.

See in D3, page 11, paragraph 3.

3.3 To meet the requirements of Rule 6.3(b) PCT the independent claim should have been properly cast in the two part form, with those features which in combination are known from D1 being placed in the preamble.

3.4 Reference signs in parentheses should have been inserted in the claims to increase their intelligibility, Rule 6.2(b) PCT. This applies to both the preamble and characterising portion.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
B30/0020		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/ 04385	23/06/1999	24/06/1998
Applicant		
TOFANI, SANTI		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

[redacted] International Application No
[redacted] T/EP 99/04385

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61N2/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	US 5 156 587 A (MONTONE LIBER J) 20 October 1992 (1992-10-20) column 1, line 10-21 column 2, line 58-68 column 5, line 37 -column 6, line 16 column 10, line 13-20 ---	3,12,13, 15 1,5,10, 16
X A	DE 39 11 393 A (KRAUS WERNER) 11 October 1990 (1990-10-11) the whole document ---	3,11-13, 15 1,2,5, 10,16
X A	DE 41 22 380 A (KRAUS WERNER) 7 January 1993 (1993-01-07) column 2, line 68 -column 3, line 22 --- -/--	3,15 1,2,16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 September 1999	06/10/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Grossmann, C.

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DE 40 36 770 A (LIFE RESONANCES, INC.) 16 May 1991 (1991-05-16) page 3, line 17 -page 4, line 28	1-3,8,9, 12,13, 15,16, 19,20
A	WO 96 39493 A (U S ENVIRONMENTAL PROTECTION A ;BLACKMAN CARL F (US); BLANCHARD JA) 12 December 1996 (1996-12-12) page 27, line 13 -page 30, line 30	1-3,8,9, 12,13, 15-17, 19,20
A	TOFANI S;, FERRARA A, ANGLESIO L, GILLI G: "evidence for genotoxic effects of resonant elf magnetic fields" BIOELECTROCHEMISTRY AND BIOENERGETICS, no. 36, 1995, page 9-13 XP002084038 cited in the application the whole document	1-3,8,9, 12,13, 15,16, 19,20
A	US 5 691 324 A (SANDYK REUVEN) 25 November 1997 (1997-11-25) column 8, line 36 -column 9, line 27 column 11, line 6-28 column 16, line 9-22 column 17, line 52 -column 18, line 33	1-5,9, 12,15-19
A	WO 97 04830 A (GRAY JAMES R) 13 February 1997 (1997-02-13) page 10, line 18-21 page 15, line 8-16 page 44, line 19 -page 47, line 12 page 52, line 15 -page 54, line 6	2,4,11, 14,15, 17,18